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Skin Autofluorescence

A tool to identify type 2 diabetic patients at risk for developing microvascular complications

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OBJECTIVE — Skin autofluorescence is a noninvasive measure of the level of tissue accumulation of advanced glycation end products, representing cumulative glycemic and oxidative stress. Recent studies have already shown a relationship between skin autofluorescence and diabetes complications, as well as the predictive value of skin autofluorescence for total and cardiovascular mortality in type 2 diabetes. Our aim was to investigate the predictive value of skin autofluorescence for the development of microvascular complications in type 2 diabetes.

RESEARCH DESIGN AND METHODS — At baseline, skin autofluorescence of 973 type 2 diabetic patients with well-controlled diabetes was noninvasively measured with an autofluorescence reader. The aggregate clinical outcome was defined as the development of any diabetes-associated microvascular complication of 881 surviving patients, which was assessed at baseline and at the end of follow-up. Single end points were the development of diabetes-associated retinopathy, neuropathy, and (micro)albuminuria.

RESULTS — After a mean follow-up period of 3.1 years, baseline skin autofluorescence was significantly higher in patients who developed any microvascular complication, neuropathy, or (micro)albuminuria but not in those who developed retinopathy. Multivariate analyses showed skin autofluorescence as a predictor for development of any microvascular complication along with A1C, for development of neuropathy along with smoking, and for development of (micro)albuminuria together with sex, A1C, and diabetes duration. Skin autofluorescence did not have predictive value for the development of retinopathy, albeit diabetes duration did.

CONCLUSIONS — Our study is the first observation of skin autofluorescence measurement as an independent predictor of development of microvascular complications in type 2 diabetes.

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Hyperglycemia, individual susceptibility, and lifestyle are three key factors that play an important role in the development of microvascular disease in diabetes. One of the consequences of hyperglycemia and the attendant increased generation of free radicals is the increased formation of advanced glycation end products (AGEs), besides the increased polyol and hexosamine fluxes and activation of protein kinase C, which all contribute to tissue damage in diabetes (1,2). Those AGEs can be described as the final products of slowly occurring nonenzymatic glycation of proteins that form cross-links with long-lived proteins such as collagen (the so-called Maillard reaction). They may also accumulate as a result of oxidative stress–related glycoxidation and lipoxidation pathways.

In the Diabetes Control and Complications Trial (DCCT), long-term intensive compared with conventional treatment of hyperglycemia in type 1 diabetic patients improved glycemic control and delayed the progression of microvascular complications (3). The UK Prospective Diabetes Study and other prospective studies have also shown an association between hyperglycemia and increased risk of microvascular complications in type 2 diabetes (4–6). The DCCT Skin Collagen Ancillary Study Group showed the association of long-term intensive treatment of hyperglycemia, as compared with conventional treatment, with lower levels of AGEs in skin collagen, and they showed that these AGE levels in skin biopsies predicted the risk of development or progression of microvascular disease in type 1 diabetes, even after adjustment for A1C (7,8).

A newly described noninvasive method to assess tissue AGEs concerns skin autofluorescence. This method is based on the specific fluorescence characteristics of AGEs and has been validated against specific AGE levels in skin biopsies in patients with diabetes or on hemodialysis and in healthy control subjects (9,10).

Recently, the relationship between skin autofluorescence, reflecting AGE accumulation, and outcome has been studied in type 2 diabetes. Besides its relation with chronic diabetes complications (in cross-sectional analyses), skin autofluorescence has also shown independent predictive value for cardiovascular mortality and morbidity in patients with type 2 diabetes and in patients with end-stage renal disease undergoing hemodialysis (10–12) In this study, we analyzed whether skin autofluorescence, as a marker of AGE accumulation, can predict the development of microvascular complications in a type 2 diabetic population.

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R.G. and A.J.S are founders of DiagnOptics B.V., Groningen, the Netherlands, manufacturer of the AGE-Reader, which is based on the prototype used in the present article.

Abbreviations: AGE, advanced glycation end product; DCCT, Diabetes Control and Complications Trial; UKPDS, UK Prospective Diabetes Study.

RESEARCH DESIGN AND METHODS

— Between May 2001 and May 2002, 973 primary care type 2 diabetic patients were included in the study cohort and had a skin autofluorescence measurement. The included patients were all participating in a shared-care project of the Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) Study and have also been described elsewhere (11). During follow-up, data of 967 patients were analyzed for this study (6 patients were lost to follow-up). Eighty-six patients died before the end of follow-up, and this subgroup will be addressed separately from the surviving 881 patients. Patients with a Fitzpatrick class V–VI skin type were excluded because of the autofluorescence reader's limitation to measure accurately in dark skin types (13–15). All participating patients visited the outpatient clinic at least once a year. Follow-up ended in January 2005. All of the included patients had given their informed consent, and approval by the local ethics committee had been obtained.

Skin autofluorescence

The autofluorescence reader (prototype of the current AGE Reader; DiagnOptics, Groningen, the Netherlands) illuminates a skin surface of ~4 cm^2, guarded against surrounding light, with an excitation light source with peak intensity at ~370 nm. Emission light and reflected excitation light from the skin is measured with a spectrometer in the 300–600 nm range, using a glass fiber. Autofluorescence was computed by dividing the average light intensity of the emission spectrum 420–600 nm by the average light intensity of the excitation spectrum 300–420 nm, multiplied by 100 and expressed in arbitrary units (AU). Skin autofluorescence of all patients was assessed at the volar side of the arm, 10 cm below the elbow fold. Six diabetes specialist nurses did the autofluorescence measurements with two identical autofluorescence reader devices. The autofluorescence reader has been validated and more extensively been described in previous studies (9,11).

Data collection

Clinical data and laboratory results were obtained at the time of the baseline skin autofluorescence measurement. Serum creatinine, nonfasting lipids (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides), and urinary albumin and creatinine were measured according to the standard laboratory procedures. A1C was measured with a Primus CLC-385 using boronate affinity chromatography and high-performance liquid chromatography (reference value 4.0–6.0%). Blood pressure measurement was a single measurement obtained after a 5-min rest with the patient in seated position, using an aneroid device. At each visit to the outpatient clinic and at the end of follow-up, the absence or presence of retinopathy, neuropathy, and (micro) albuminuria was assessed.

Clinical end points

The aggregate clinical end point was the development of any diabetes-associated microvascular complication, which was defined as the presence of at least one of the following diabetes complications according to the American Diabetes Association definitions: retinopathy, neuropathy, and/or (micro)albuminuria (16). The single clinical end points were described as the development of retinopathy, neuropathy, or (micro)albuminuria. Retinopathy was determined by an ophthalmologist based on retinal photography. Presence of at least background retinopathy was assumed to imply retinopathy. Neuropathy was examined using a 5.07/10 g Semmes-Weinstein monofilament, applied on the dorsum of both feet at three different, noncallused areas (first toe and first and fifth distal metatarsal bone). Neuropathy was considered in cases of diminished sensibility, which was defined as at least two incorrect responses after three applications at each area (two real and one false application) (17,18). (Micro)albuminuria at baseline was defined as an albumin-to-creatinine ratio >2.5 mg/mmol for men and >3.5 mg/mmol for women in two subsequent urine samples or once in the year before baseline while using an ACE inhibitor at baseline (19). Newly developed (micro) albuminuria at follow-up was defined as an albumin-to-creatinine ratio >2.5 mg/mmol for men and >3.5 mg/mmol for women in two urine samples (one in the year before and one at the moment during follow-up) or an abnormal level of the albumin-to-creatinine ratio in the year before the end of follow-up while using an ACE inhibitor at follow-up.

Statistical analysis

One-way ANOVA using post hoc multiple comparisons (with Bonferroni correction) was used to compare mean skin autofluorescence between subgroups of microvascular complications in the 881 surviving patients. Subgroups are as follows: *1*) no microvascular complication at baseline or at follow-up, *2*) no microvascular complication at baseline but a microvascular complication at follow-up, and *C*) a microvascular complication at baseline and at follow-up.

Univariate and multivariate multinominal regression analyses were performed to determine the relationship of skin autofluorescence to the presence or development of microvascular disease. Patients without signs of microvascular complications at baseline or at follow-up formed the reference categories in these calculations. In the multivariate analyses, we controlled for potential confounding risk factors for the development of microvascular complications, which were derived from the UKPDS findings, including sex, diabetes duration, A1C, current smoking, systolic blood pressure, HDL cholesterol, LDL cholesterol, and triglycerides, with the addition of BMI (4).

Odds ratios (ORs) (95% CI) for skin autofluorescence were calculated in the univariate and multivariate analyses. *P* values <0.05 were considered statistically significant.

RESULTS

— The baseline characteristics of the surviving study population including mean skin autofluorescence of the total group are shown in Table 1. Mean age of our study population was 66 years, 46% of whom were male, with a relatively short median diabetes duration of 4.0 years (interquartile range 1.5–8.1). Eighty-five percent of this study population with well-controlled diabetes was on a diet and/or oral agents; the other 15% of patients received insulin or combined insulin/oral agent treatment. In the 881 survivors, the prevalence of retinopathy, neuropathy, and (micro)albuminuria at baseline was 19, 24, and 24%, respectively, resulting in an overall percentage of patients with diabetes-associated microvascular complication of 50%.

Table 2 shows the mean baseline skin autofluorescence of the 881 survivors subdivided in groups with continued absence or presence or the development of microvascular complications at follow-up. During a median follow-up period of 3.1 years, 61 patients (7.0%) developed retinopathy; their baseline skin autofluorescence did not differ from skin autofluorescence levels of patients who did not show or already had retinopathy

Table 1—*Characteristics of the type 2 diabetic patients*

Characteristic	
n	881
Age (years)	66 ± 11
Sex (male/female)	406/475
Smoking (%)	19
BMI (kg/m²)	29.4 ± 4.8
Systolic blood pressure (mmHg)	146 ± 20
Diabetes duration (years)	4.0 (1.5–8.1)
A1C (%)	6.6 (6.0–7.6)
Creatinine (μmol/l)	95 ± 19
Creatinine clearance (ml/min)	77 ± 27
Urinary albumin-to-creatinine ratio	1.41 (0.76–3.79)
Total cholesterol (mmol/l)	5.2 ± 1.0
HDL cholesterol (mmol/l)	1.3 ± 0.3
LDL cholesterol (mmol/l)	2.9 ± 0.9
Triglycerides in mmol/l	2.1 (1.4–2.9)
Microvascular disease (%)	50
Retinopathy	19
Neuropathy	24
(Micro)albuminuria	24
Macrovascular disease (%)	37
Skin autofluorescence (total group) (AU)	2.74 ± 0.7

Values are means ± SD or median (interquartile range) unless otherwise indicated. Reference values of the laboratory: A1C 4.0–6.0%, creatinine 70–110 μmol/l, creatinine clearance (Cockcroft-Gault formula) 80–120 ml/min, urinary albumin-to-creatinine ratio 0–2.5, total cholesterol 3.5–5.0 mmol/l, HDL cholesterol 0.9–1.7 mmol/l, LDL cholesterol 3.6–4.4 mmol/l, and triglycerides 0.6–2.2 mmol/l.

at baseline. However, skin autofluorescence was higher in the patient groups who developed neuropathy or (micro)albuminuria compared with that in patients without these complications. At follow-up, newly developed neuropathy was diagnosed in 7.5% and newly developed (micro)albuminuria in 10.1% of patients; 12.5% of the population developed at least one microvascular complication. Skin autofluorescence at baseline was also significantly higher in the patient groups that developed any microvascular complication or who already had a microvascular complication at baseline compared with patients who did not develop any microvascular disease.

Multinominal logistic regression analysis showed that skin autofluorescence was a strong predictor of the development of the aggregate of microvascular complications (OR 2.05 [95% CI 1.51–2.80], $P < 0.001$). Skin autofluorescence was significantly associated with the development of retinopathy (1.42 [1.01–1.99], $P = 0.042$), neuropathy (1.59 [1.15–2.19], $P = 0.005$), and (micro)albuminuria (1.73 [1.28–2.34], $P < 0.001$). After correction for the confounding risk factors, baseline skin autofluorescence still appeared to be significantly associated with the development of these end points, except for retinopathy (1.21 [0.83–1.74], $P = 0.32$) (Table 3). Diabetes duration at baseline was the only significant independent variable for development of retinopathy in this multivariate analysis (1.10 [1.06–1.15], $P < 0.001$). Surviving smokers less often developed neuropathy compared with nonsmokers. In the nonsurviving group (86 patients), 70% had a microvascular complication at baseline; there were 23 nonsurviving smokers. Seventy percent of the nonsurviving smokers already had a microvascular complication at baseline, and 13% of the nonsurviving smokers developed a microvascular complication before they died.

When baseline skin autofluorescence levels are categorized in subgroups of practically feasible levels of skin autofluorescence (three categories in rounded tertiles: skin autofluorescence <2.35 AU, 2.35 ≤ skin autofluorescence <3.00 AU, and skin autofluorescence ≥3.00 AU); those in the category skin autofluorescence ≥3.00 AU do have a higher chance to develop a microvascular complication compared with patients with a lower skin autofluorescence level (Table 4).

Table 2—*Mean ± SD skin autofluorescence at baseline and mean differences between groups*

	A: t_0 absent/t_{fu} absent	B: t_0 absent/t_{fu} present	C: t_0 present/t_{fu} present	B vs. A	C vs. A	C vs. B
Microvascular complication						
Retinopathy	2.69 ± 0.73	2.88 ± 0.74	2.91 ± 0.72	0.20	0.22	0.02
n	647	61	169	(−0.04 to 0.43)	(0.07–0.37)	(−0.24 to 0.29)
P				0.14	0.002	1.00
Neuropathy	2.67 ± 0.72	2.93 ± 0.75	2.88 ± 0.75	0.26	0.21	−0.05
n	596	66	215	(0.03–0.49)	(0.07–0.35)	(−0.29 to 0.20)
P				0.019	0.001	1.00
(Micro)albuminuria	2.62 ± 0.68	2.91 ± 0.67	2.97 ± 0.83	0.28	0.34	0.06
n	570	87	207	(0.09–0.48)	(0.20–0.48)	(−0.16 to 0.28)
P				0.002	<0.001	1.00
Any	2.52 ± 0.69	2.86 ± 0.66	2.88 ± 0.75	0.34	0.36	0.01
n	322	109	441	(0.15–0.53)	(0.23–0.48)	(−0.17 to 0.20)
P				<0.001	<0.001	1.00

Data are means ± SD of skin autofluorescence in AUs within the group or mean differences between groups (95% CI) (ANOVA with Bonferroni correction). t_0, baseline; t_{fu}, follow-up.

Table 3—*Variables related to the development of microvascular complications in type 2 diabetes by multinominal logistic regression analysis*

	Any microvascular complication		Retinopathy		Neuropathy		(Micro)albuminuria	
Variables	*P*	OR (95% CI)	*P*	OR (95% CI	*P*	OR (95% CI)	*P*	OR (95% CI)
Skin AF	<0.001	2.02 (1.45–2.81)	0.32	1.21 (0.83–1.74)	0.026	1.50 (1.05–2.14)	<0.001	1.88 (1.36–2.61)
Sex	0.02	0.55 (0.33–0.90)	0.91	0.97 (0.53–1.75)	0.78	1.09 (0.61–1.93)	0.001	0.42 (0.25–0.71)
A1C	0.004	1.30 (1.09–1.55)	0.13	1.18 (0.95–1.45)	0.87	1.02 (0.82–1.26)	0.034	1.21 (1.01–1.44)
Diabetes duration	0.66	1.01 (0.96–1.06)	<0.001	1.10 (1.06–1.15)	0.032	1.04 (1.00–1.08)	0.04	0.95 (0.90–0.997)
Smoking	0.07	0.56 (0.29–1.05)	0.09	0.48 (0.21–1.11)	0.011	0.29 (0.11–0.75)	0.96	1.02 (0.56–1.85)
Systolic blood pressure	0.43	1.01 (0.99–1.02)	0.39	1.01 (0.99–1.02)	0.49	1.01 (0.99–1.02)	0.18	1.01 (0.996–1.02)
LDL cholesterol	0.48	1.09 (0.85–1.40)	0.66	0.93 (0.69–1.27)	0.35	0.87 (0.64–1.17)	0.30	1.15 (0.89–1.49)
HDL cholesterol	0.26	0.62 (0.27–1.43)	0.36	0.63 (0.23–1.70)	0.081	0.41 (0.15–1.12)	0.40	0.38 (0.15–0.96)
Triglycerides	0.54	0.94 (0.78–1.14)	0.41	0.91 (0.72–1.15)	0.85	0.98 (0.79–1.22)	0.19	0.87 (0.71–1.07)
BMI	0.27	1.03 (0.98–1.08)	0.33	1.03 (0.97–1.09)	0.56	0.98 (0.93–1.04)	0.39	1.02 (0.97–1.08)

AF, autofluorescence measured with the autofluorescence reader (see RESEARCH DESIGN AND METHODS).

CONCLUSIONS — Our study provides the first evidence that skin autofluorescence is an independent predictor of development of microvascular complications in a population of patients with well-controlled type 2 diabetes. Separately, this also holds for the development of neuropathy and (micro)albuminuria (and in univariate analysis for retinopathy). This noninvasive marker of tissue AGE accumulation may reflect the deleterious effects of long-term glycemic and oxidative stress. Meerwaldt et al. (12) recently showed that skin autofluorescence is a predictor of 5-year coronary heart disease and mortality in diabetes. The present study shows that skin autofluorescence also has a predictive value for the development of microvascular complications that, in the analysis of this study, is superior to that of many other commonly used risk predictors, such as diabetes duration and A1C, in type 2 diabetes. This conclusion is applicable for primary care type 2 diabetic patients treated according to current standards, which is the large majority of type 2 diabetes patients in the Netherlands.

The DCCT/EDIC (Epidemiology of Diabetes Interventions and Complications) substudy already showed the predictive value for skin AGE levels obtained from skin biopsies for the progression of microvascular complications in patients with type 1 diabetes (8). Our study population consisted of type 2 diabetic patients with skin AGE level assessment by means of a noninvasive, rapid method. Another difference is that the DCCT/EDIC substudy investigated the development as well as the progression of microvascular complications. The limited follow-up period; the low rate of clearly classifiable progression of the microvascular complications, especially retinopathy; and the confounding role of introduced medication made us decide to restrict our study to the evaluation of the development of microvascular complications and not to address progression of these diabetes complications.

In retinopathy, skin autofluorescence turned out to have no prognostic value in the multivariate analysis. Possible explanations are the short follow-up period and the smaller amount of patients who developed retinopathy versus the other complications. Moreover, the different pathophysiologic mechanisms of microvascular damage in the different organs (retina, kidneys, and neurons) could play a role in the differences in incidence rates of outcomes. In particular, the pathobiology of retinopathy might be different from that of the kidney and neurologic system as a result of a different role of vascular endothelial growth factor as a possible mediator for proliferation (20).

(Micro)albuminuria is an early clinical sign of diabetic nephropathy; when left untreated, it predicts a high risk for the development of progressive renal damage, which eventually may lead to end-stage renal disease. Progressive renal disease is also associated with a vastly increased cardiovascular risk. This study defined (micro)albuminuria as a sign of microvascular complications with the intention to reflect early stages of diabetic nephropathy.

In the predictive analyses, the nonsurviving patients were excluded from the analyses. These nonsurvivors had markedly increased skin autofluorescence values, but they also had a very high prevalence of microvascular complications at baseline (70%), so this does not reduce the strength of the relation between skin autofluorescence and microvascular complications.

Ethnicity is one of the mentioned UKPDS confounding risk factors for the development of microvascular disease. Because of the limitation of measuring skin autofluorescence in dark skin types associated with the prototype of the AGE

Table 4—*Prediction of newly developed microvascular complications subdivided into three skin autofluorescence (AF) groups*

Microvascular complication	*n**	Skin AF <2.35 AU	2.35 ≤ Skin AF <3.00 AU	Skin AF ≥3.00 AU
Retinopathy	708	15/241 (6.2)	18/251 (7.2)	28/216 (13.0)
Neuropathy	662	11/219 (5.0)	27/247 (10.9)	28/196 (14.3)
(Micro)albuminuria	657	18/225 (8.0)	31/253 (12.3)	38/179 (21.2)
Any	431	23/161 (14.3)	41/167 (24.6)	45/103 (43.7)

Data are *n* (%) of newly developed microvascular complications of subgroups compared with the group who did not develop a microvascular complication. *Patients who did not have a complication at baseline. Subgroups of skin AF are tertiles rounded to a practical level.

reader used in the present study, individuals with dark skin had to be excluded. Over 95% of the participants were Caucasian; therefore, ethnicity was not taken into account in the analyses. Further developments of the AGE reader may hopefully enable measurements in dark skin type in future investigations.

Lutgers et al. (11) previously described the other limitations of the autofluorescence reader as a marker of tissue AGE accumulation: nonfluorescent AGEs will not be measured with the autofluorescence reader, and other tissue components that fluoresce in the same range of wavelength might be confounders.

In conclusion, our study confirms skin autofluorescence as a helpful clinical method to identify type 2 diabetic patients at risk for developing any microvascular complication, neuropathy, and (micro)albuminuria. Further investigation with longer follow-up needs to be done to assess whether skin autofluorescence is a factor in the development of diabetic retinopathy and to assess the relationship of skin autofluorescence and the progression of microvascular complications. Its noninvasive and time-saving application makes the autofluorescence reader an easy clinical tool that is useful in the outpatient clinic in risk assessment and for monitoring changes in accumulation of tissue AGEs reflecting long-term glycemic stress.

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